GENAXXON bioscience
Tel: +49(0)731 – 3608 123
Fax: +49(0)731 – 3608 962
E-Mail: info@genaxxon.com

SNP PolTaq DNA Polymerase with Buffer

Component	Cat#	M3025.0250	M3025.1000	Colour code of cap
SNP PolTaq DNA Polymerase (5 units/µL)		250 units	1x 1000 units	**colourless**
10X buffer for SNP Pol DNA Polymerase		1.25 mL	2x 1.25mL	**colourless**

Product description

SNP PolTaq DNA polymerase (High Discrimination DNA Polymerase) is a highly selective DNA polymerase variant with hotstart properties. It has been selected for assays in which High Discrimination is required, for instance in allele-specific PCRs or methylation-specific PCRs. Whereas many DNA polymerases tolerate mismatched primer-template complexes, SNP PolTaq DNA polymerase efficiently discriminates those and only produces specific amplicons in case of perfectly matched primer pairs. This makes SNP PolTaq DNA polymerase very useful for SNP detections, HLA genotyping or the analysis of single CpG methylation sites.

SNP PolTaq DNA polymerase efficiently discriminates primers, which have a mismatch at the 3'-end.

The primer with the mismatch at the 3'-position is the absolute requirement for discrimination of SNPs. If the mutation/mismatch is situated at another place of the primer SNP Pol and SNP PolTaq polymerase will work just as a "normal" Taq polymerase.

The engineered polymerase is also available as SNP Pol DNA polymerase (M3009) version without a 5'-3'-nuclease activity that cannot be used for hydrolysis probe-based assays (Taqman® probes, molecular Beacons, etc.)!

Attention: The SNP PolTaq is an aptamer inhibited hot start DNA polymerase. The aptamer oligo used reversibly inhibits the polymerase at temperatures <55°C! Therefore, the primers should ideally have an annealing temperature of >57°C.
Please design your primers accordingly. You can also include LNA-modified oligos to increase the annealing temperature. The LNA modification should ideally be in the middle of the primer.

Product Specifications

Concentration:	5 units/µL
Substrate analogues:	dNTP, ddNTP, fluorescent dNTP/ddNTP
Extension rate:	0.5 to 1-kb/min. at 72°C
5'-3' exonuclease activity:	No for SNP Pol Polymerase / Yes for SNP PolTaq Polymerase)
3'-5' exonuclease activity:	No
Nuclease contamination:	No
Protease contamination:	No

Unit definition

One unit of SNP Pol / SNP PolTaq DNA-Polymerase is defined as the amount of enzyme that incorporates 10nmol of dNTP's into acid-insoluble fraction in 30 minutes at 72°C under standard assay conditions.

Quality Control

SNP Pol DNA polymerase is tested for successful ASA performance detecting a genomic SNP (rs72921001) in HeLa genomic DNA. The activity of SNP Pol DNA polymerase is monitored and adjusted to a specific DNA polymerase activity using an artificial DNA template and a DNA primer.
Enzyme concentration is determined by protein-specific staining.
Please inquire more information at info@genaxxon.com for the lot-specific concentration
No contamination has been detected in standard test reactions.

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U.St.-Id.Nr. DE 220 603 767 ● Deutsche St.-Nr. 88002/33658

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Application

- SNP-detection by allele-specific amplification (ASA) / Allele-specific PCR
- Genotyping and genomic profiling
- Methylation specific PCR (MSP)
- HLA genotyping
- Multiplex PCR

Stability

Genaxxon bioscience SNP Pol and SNP PolTaq DNA-Polymerase is shipped on wet ice but retain full activity at RT (+15°C to +25°C) for at least 2 weeks.

SNP Pol and SNP PolTaq DNA Polymerase, including buffers and reagents, should be stored immediately upon receipt at -20°C. When stored under these conditions and handled correctly, these products can be kept at least until the expiration date (see tube label) without showing any reduction in performance. The Genaxxon bioscience SNP Pol and SNP PolTaq DNA Polymerase can also be stored at +2°C - +8°C up to 9 months.

Product Use Limitations

The product (SNP Pol DNA Polymerase and SNP PolTaq DNA Polymerase) is covered by a pending patent application. It is the purchaser's own internal research use and may not be resold, modified or used for production and commercial purposes of any kind.

For information on obtaining additional rights, please contact Genaxxon bioscience (info@genaxxon.com).

This product is for research use only and may be used in vitro experiments only.

Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.

This product does not require a Material Safety Data Sheet because it does neither contain more than 1% of a component classified as dangerous or hazardous nor more than 0.1% of a component classified as carcinogenic. However, we generally recommend, when working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.

Genaxxon bioscience takes no liability for damage resulting from handling or contact with this product.

More information can be found in the REGULATION (EC) No. 1272/2008 OF THE EUROPEAN PARLIAMENT AND THE COUNCIL or contact Genaxxon bioscience (info@genaxxon.com)

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PCR Protocol Part

SNP PolTaq DNA Polymerase PCR Protocol

This protocol serves as a guideline for PCR amplification. Optimal reaction conditions such as incubation times, temperatures, and amount of template DNA may vary and must be determined individually.

Important notes before getting started

- Set up all reaction mixtures in an area separate from that used for DNA preparation or PCR product analysis.
- Spin down and mix all solutions carefully before use.
- SNP PolTaq 10X buffer is optimized for short amplicon length (about 60 – 200bp), but also longer amplicons are possible. The addition of additional MgCl2 (0.5 – 1.5mM) might be needed in case of longer amplicons (>500bp).
- SNP PolTaq DNA polymerase can also be used for realtime cycling when adding a suitable realtime PCR fluorescence dye.
- SNP PolTaq DNA polymerase can be used together with probe-based assays.
- Use disposable tips containing hydrophobic filters to minimize cross-contamination.

Things to do before starting

- If required, prepare a dNTP-mix containing 10mM of each dNTP (or ready-to-use product M3016 from Genaxxon bioscience). Store this mix in aliquots at -20°C. For your convenience, the SNP Pol 2X Master Mix (M3061) already contains a premixed solution containing SNP Pol DNA Polymerase, buffer, and dNTPs.

Procedure

1. **Thaw 10X buffer, dNTPs or dNTP-mix, primer solutions, and 25mM MgCl2** (if required) at RT or on ice.
   Keep the solutions on ice after complete thawing. Mix well before use to avoid localized differences in salt concentration.
2. **Prepare a master mix according to Table 1.**
   The master mix typically contains all of the components needed for PCR except the template DNA. Prepare a volume of master mix 10% greater than that required for the total number of PCR assays to be performed. A negative control (without template DNA) should always be included in every experiment.
   Keep the master mix on ice.

Table 1: Recommendations for PCR and qPCR / Reaction Setup (50µL PCR reaction)

Components	Volume	Final concentration
SNP PolTaq DNA Polymerase	0.5µL	2.5 units/50µL reaction
Nucleotides	1µL	of a 10mM ready-to-use dNTP mix (M3016).
10X amplification buffer	5µL	1X buffer
primer forward (10µM)*:	1µL	0.2 µM (0.05 – 1.0µM)
primer reverse (10µM):*	1µL	0.2 µM (0.05 – 1.0µM)
Probe**	x µL	0.2µM (0.05 – 0.3µM)
Template DNA / sample extract	y µL	<10ng plasmid DNA or <500ng genomic DNA
sterile, bidestilled water	z µL	up to 50µL total volume

Keep all components on ice.
Spin down and mix all solutions carefully before use.
* Primers should ideally have a GC content of 40-60%. For optimal results we recommend amplicon lengths in the range of 60 to 300bp.
** The necessary concentration of probe depends very much on the probe sequence and the kind of probe. Please test for optimum!

This product is compatible for the use with hydrolysis probe systems, e.g. TaqMan probes and qPCR cycler not requiring a passive reference dye!

Table 2: Final MgCl2 concentration in a **50µL reaction**

Additional MgCl2 conc. in reaction (mM)	0.5	1.0	1.5	2.0
Additional volume (µL) of 25mM MgCl2 per 50µL reaction.	1	2	3	4

Note: The Mg2+ concentration provided by the supplied SNP PolTaq PCR-buffer will produce satisfactory results in most cases. However, in some cases, e.g., amplicon size >500bp reactions may be improved by increasing the final Mg2+ concentration according to Table 2.

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3. **Mix the master mix thoroughly** and dispense appropriate volumes into PCR tubes.
   Mix gently, for example, by pipetting the master mix up and down. It is recommended that PCR tubes are kept on ice before placing in the thermal cycler.
4. **Add template DNA** to the individual tubes containing the master mix.
   For RT-PCR, add an aliquot from the reverse transcriptase reaction. The volume added should not exceed 10% of final PCR volume.
5. **Program the thermal cycler** according to the manufacturer's instructions.
   A typical PCR cycling program is outlined in Table 3. For maximum yield and specificity, temperatures and cycling times should be optimized for each new target or primer pair.

Table 3: PCR conditions (**Thermal cycler**)

Step	time	temperature	comments
Initial denaturation:	2 min.	95°C	
3-step cycling			
Denaturation:	15 sec.	95°C	
Annealing:	10 sec.	>55°C *	approximately 3°C to 5°C* below lower Tm of primers.
Extension:	30 sec./250bp	72°C	approx. 0.5kbp per minute extension rate
Number of Cycles	25 - 40		
Hold		<10°C	

Note: After amplification, samples can be stored at +2°C to +8°C overnight, or -20°C for long term storage.
*** Note:** The SNP Pol is an aptamer inhibited hot start DNA polymerase. The aptamer oligo used reversibly inhibits the polymerase at temperatures <55°C! Therefore, the primers should ideally have an annealing temperature of >57°C. Please design your primers accordingly.

Table 4: Recommendations for Standard PCR-Primers

Length:	18-30 nucleotides
GC-Content:	40-60%
Tm:	Design primer pairs with similar Tm values.
	Optimal annealing temperature may be above OR below the estimated Tm. As a starting point, use an annealing temperature of 3°C to 5°C below Tm of the primer with the lower Tm-Value.
Sequence:	Avoid complementarities of two or more bases at the 3' ends of primer pairs.
	Avoid runs of 3 or more Gs or Cs at the 3' end.
	Avoid a 3'-end T.
	Avoid complementary sequences within primer and between primer pairs.

Dye in agarose gel	0.5%-1.5%	2.0%-3.0%	CAS-number	Cat-No. Genaxxon
Xylene cyanol	10000bp - 4000bp	750bp - 200bp	2650-17-1	M3312
Cresol Red	2000bp - 1000bp	200bp - 125bp	62625-29-0	M3371
Bromophenol blue	500bp - 400bp	150bp - 50bp	115-39-9	M3092
Orange G	<100bp	<20bp	1936-15-8	M3180
Tartrazine	<20bp	<20bp	1934-21-0	

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